

Tracking Released Japanese Flounder *Paralichthys olivaceus* by Mitochondrial DNA Sequence

Tetsuo Fujii

Japan Sea National Fisheries Research Institute

Fisheries Research Agency

1-5939-22, Suido-cho, Niigata 951-8121

JAPAN

Email: tefujii@affrc.go.jp

Key Words: Japanese flounder, migration, genetic diversity, mtDNA tag

Abstract

A method of tracking released Japanese flounder *Paralichthys olivaceus* was developed by using the extremely high sequence variability of the mitochondrial DNA, particularly in the control region. The determination of the mother of a released flounder is possible by sequencing the mitochondrial DNA control region because of its variability and maternal inheritance. In this method, sequences of hatchery-reared juveniles should be analyzed first. The sequence data are then registered in a database, so that when the flounder is later caught its origin can be determined. We can obtain information about the genetic variability of hatchery-reared stocks in the sea, as well as the migration of the released flounder. Although other methods have been used to track released flounder, mitochondrial DNA sequence appears to be less harmful for juveniles and potentially less expensive.

The Japanese flounder *Paralichthys olivaceus* is one of the most important species for the coastal fisheries in Japan. More than 30 million hatchery-reared juveniles have been released annually for the enhancement of stocks. It is well known that the wild flounder migrate widely as they grow (Minami, 1997). To elucidate the migration of the released flounder it is necessary to carefully manage the genetics as well as evaluate stocking effectiveness. Previous tagging studies have been performed using external tags or chemical markings. External tags may be harmful to juveniles (most juveniles are released less than 10 cm in total length) and difficult to place on large numbers of fish. On the other hand, chemical markings, such as fluorescent marking in otoliths, are comparably easy to apply, but the patterns of the marks are limited. To avoid these tagging obstacles, a method of tracking released Japanese flounder by mitochondrial DNA (mtDNA) sequence was developed. In this paper, the concept and advantages of the mtDNA tag are described. Potentials for use of the mtDNA tag and future programs are also introduced briefly.

Concept of mtDNA Tag

In this method, sequences of hatchery-produced juveniles must first be analyzed and registered in a database. The mtDNA of wild Japanese flounder is characterized by the extremely high sequence variability especially in the control region (D-loop region). The direct sequencing of the 350 base pairs (bp) in the first half of the control region showed that 126 sites (36.0%) were variable. The sequence differences between individuals were up to 8.3%, with an average

of 4.3%, as 54 haplotypes were detected from 55 individuals (Fujii and Nishida, 1997). This means that almost all flounder have their own unique sequences and mothers of hatchery-reared juveniles can be determined because of its high variability and maternal inheritance. In Japan there is at least one hatchery in every prefecture, each having its own broodstock. This makes hatchery-rearing of juveniles localized. Since most of the hatcheries are supported by the prefectures, most juveniles are released in their home prefectures. When the released flounder are recaptured later, their origins of rearing and release can be determined by their mtDNA sequences.

Fortunately, most of hatchery-reared juveniles have abnormal pigmentation, called melanism, on their blind sides. Therefore it is easy to distinguish released flounder from wild ones. DNA analysis is also possible from a single fish scale preserved in 99.5% ethanol. All that would be necessary at the fish market is to pick one scale from each flounder having melanism on the blind side.

Advantages of the mtDNA Tag

The mitochondrial DNA tag has the following advantages:

- The DNA tag will never change or detach.
- DNA analysis is possible from a single fish scale, which is less harmful to the fish and reduces the cost of purchasing them to check for tags.
- It is possible to obtain data about individual fish.
- The cost for the DNA tag does not depend on the number of fish released, but rather on the number of haplotypes present in hatchery-reared juveniles and the number of recaptured fish. It costs approximately \$2.50 per sample of muscular tissue and \$3.25 for scales, including reagents, tubes and tips, and maintenance fee for gears.
- One sample may be analyzed in less than one day, and up to 200 samples may be analyzed per week.
- It is possible to get data about genetic diversity of both hatchery-reared juveniles and recaptured fish as a haplotypic diversity of mtDNA.

In conclusion, the mtDNA tag method is immutable, easy to sample, easy to analyze, economical and multipurpose.

Potential of mtDNA Tag

The efficiency of the mtDNA tag relies on high sequence variability and distinctive appearance between wild and released fish. The mtDNA sequence of the red sea bream *Pagrus major*, one of the most important species used in Japanese stock enhancement programs, is highly variable (Tabata and Mizuta, 1997) and easily distinguished from wild fish by the lack of an inter-nostril epidermis (Yamazaki, 1998). The mtDNA tag is therefore considered a useful tool for tracking hatchery-reared red sea bream. Differences in appearance between wild and hatchery-reared fish enable scientists to analyze more fish and determine their origins.

Future Programs

We have just begun the project to elucidate the migration of released Japanese flounder along the Sea of Japan under the cooperation of prefectural staffs. Preliminary results showed that released Japanese flounder began to migrate in their second winter mainly against the Tsushima current, which flows from the southwest to northeast.

Some of them migrated more than 300 km and flounder from several hatcheries were recaptured together in many regions. The haplotypic diversity of a group of recaptured fish is usually higher than those released from a single hatchery, due to a mixture of flounder released from several hatcheries and their conserved genetic diversity until recaptured. The mitochondrial DNA tag is useful not only for the elucidation of the migration but also for the monitoring of genetic diversity. It is expected that these methods will be put into effect in the near future.

Literature Cited

- Fujii, T. and M. Nishida.** 1997. High sequence variability in the mitochondrial DNA control region of the Japanese flounder *Paralichthys olivaceus*. *Fish. Sci.* 63:906-910.
- Minami, T.** 1997. Life History, Biology and Stock Enhancement of Japanese Flounder Koseisha Koseikaku, Tokyo, pp 9-24 (In Japanese).
- Tabata, K. and A. Mizuta.** 1997. RFLP analysis of the mtDNA D-loop region in red sea bream *Pagrus major* population from four locations of western Japan. *Fish. Sci.* 63:211-217.
- Yamazaki, A.** 1997. Deformities of the pectoral fin and inter-nostril epidermis and the discrimination of the artificially produced and wild red sea bream *Pagrus major* in release-recapture research. *Saibai Giken* 26:61-65 (In Japanese).